III.8—BACTERIAL PRODUCTION STIMULATED ACROSS THE ZONE OF INFLUENCE OF A GROUNDWATER CIRCULATION WELL IN A BTEX-CONTAMINATED AQUIFER

Michael T. Montgomery, Thomas J. Boyd, Barry J. Spargo,
James G. Mueller, Richard B. Coffin, and David C. Smith

INTRODUCTION

In fuel-contaminated groundwater, benzene, toluene, ethylbenzene, and xylene (BTEX) hydrocarbons are often the most abundant carbon source for bacteria. In situ bioremediation strategies often involve stimulating overall production of the natural bacterial assemblage to increase their carbon demand. Hydrocarbons may eventually be biodegraded as a result of this stimulation. In this study, groundwater circulation wells (GCWs) were used as an in situ treatment for a fuel-contaminated aquifer. GCWs may stimulate bacterial production by circulating limiting nutrients, enhancing bioavailability of hydrocarbons, or by removing inhibitory low-molecular-weight compounds through abiotic removal (e.g., air stripping). Changes in bacterial abundance were not a useful parameter to evaluate stimulation of the natural assemblage resulting from the well operation. Production of the heterotrophic bacterial assemblage was measured by rates of bacterial protein synthesis (leucine incorporation). We found that bacterial production was stimulated across the zone of influence of a series of GCWs placed in a brackish aquifer at the fuel-contaminated site. Bacterial productivity increased from 10^3 to over 10^6 cells mL^{-1} h^{-1} across the zones of influence of the main GCWs and biocurtain suggesting that this treatment stimulates overall biodegradation of carbon sources present in the groundwater. Maximum stimulation of heterotrophic activity within the main well after 9 months of operation resulted in turnover times of the free-living assemblage of around 1 h, which is extremely rapid for groundwater environments. While bacterial productivity measurements alone cannot determine the effectiveness of a bioremediation treatment, they can be a sensitive tool for evaluating overall changes in the growth state of the natural assemblage. This information is often critical in evaluating performance of a given bioremediation strategy.

The basic concept behind in situ bioremediation strategies is that they stimulate overall production of the natural bacterial assemblage. Increasing overall bacterial production increases the carbon demand at the treated site and, subsequently, fuel hydrocarbons are biodegraded as a carbon source. Typical bioremediation studies show an increase in bacterial abundance or standing stock (plate counts) as evidence of biodegradation. However, in this study, we evaluated the effectiveness of GCWs in stimulating bacterial activity, using bacterial production measurements. Though this radioassay is somewhat more difficult to perform, it is far more sensitive and less misleading in determining the growth state of the natural assemblage. In this study, production of the bacterial assemblage was measured by rates of leucine incorporation into bacterial protein synthesis [Montgomery et al., Chapter II.8) [3].

MATERIALS AND METHODS

Site Description

In December 1984, leaded and unleaded gasolines were discovered in an unconfined, semiperched aquifer adjacent to the Navy Exchange Gasoline Center (NEX) located at the Naval Construction Battalion Command (NCBC) in Port Hueneme, California (Fig. 1). Tests performed in March 1985 indicated that gasoline was leaking from two of the fuel lines connecting the storage tanks to the pumps. It was estimated that between September 1984 and March 1985, 10,800 gal of gasoline containing methyl tertiary butyl ether (MTBE) and 1,2-dichloroethane (DCA) had leaked into the subsurface. New underground storage tanks and fuel supply lines were installed immediately after verification of the spill. Over 90% of the site is covered by asphalt, so rainfall infiltration is minimized.

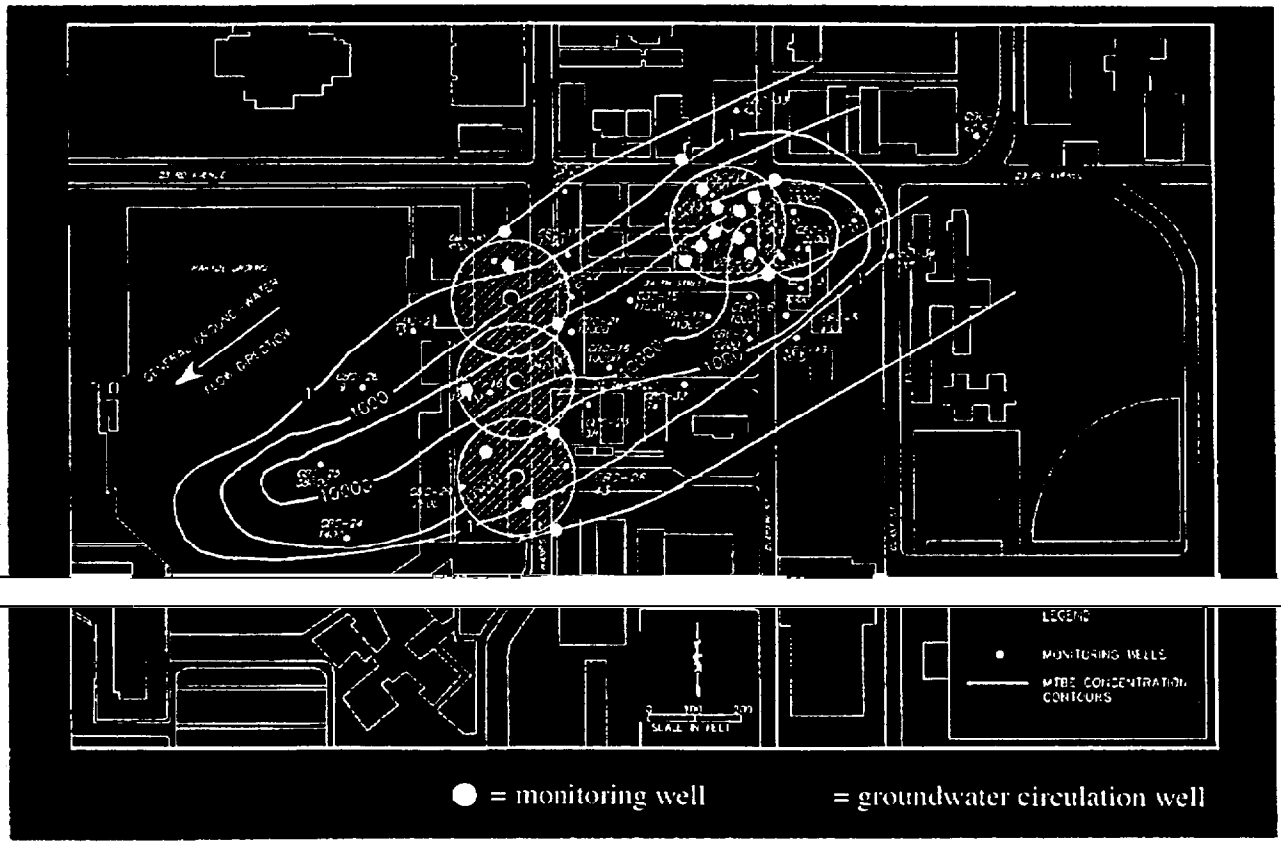

Fig. 1 — Monitoring well locations at the BTEX-contaminated site in Port Hueneme

The water table lies 2.4 to 3 m below the ground surface. Water flow is estimated at 0.3 m per day (toward the southwest) and is thought to discharge into drainage ditches, harbors, and beaches near NCBC. The water is considered brackish-to-saline and, consequently, not used as a well water supply. A clay aquitard, ranging in thickness from 6.1 to 15 m forms the lower boundary of the semiperched aquifer. Below this lie two aquifer systems separated by nonconformity. An upward hydraulic gradient exists between the upper aquifer system and the clay aquitard. Water supply wells penetrating this system are located approximately 1 mi northeast of the spill site.

Twelve monitoring wells (MW-1 to -12) were placed in an array radiating from the main treatment well in the center of the gasoline plume with an additional eight monitoring wells (MW-13 to -20) around the three smaller GCWs that formed the biocurtain. Groundwater flowed from MW-1 across the main well and through the biocurtain toward MW-15 (Fig. 1).

Bacterial Abundance

Bacterial abundance was measured by acridine orange staining and epifluorescence microscopy (acridine orange direct count (AODC)) [1,2]. Total heterotrophic plate counts (THPC) were also used to determine bacterial abundance.

Bacterial Productivity

Bacterial productivity was determined by the leucine incorporation method [3] as adapted by Smith and Azam [4]. Briefly, 5.1 μL of L-[4,5-^{3}H] Leucine (^{3}H-Leu;spec. act. = 1.54-1.58 Ci mmol^{-1}; Amersham, Arlington Heights, Illinois) was added into sterile 2 mL capacity screw cap microfuge tubes with O-rings (Fisher, Pittsburgh, Pennsylvania) prior to the addition of 1.7 mL of groundwater. Trichloroacetic acid (TCA) was added to blanks (final = 5%) and to end incubations. Samples were incubated at 21° C for 2 h to allow incorporation of radio label into bacteria and then incubated an additional 30 min after addition of TCA (21° C) prior to centrifugation (16 000 × g, 10 min, Eppendorf, 5415C). Supernate was removed by aspiration, and the pellet was washed with 1.5 mL of TCA (5%), the samples centrifuged (respun), and aspirated. The pellets were then washed with 1 mL of ethanol (80%), respun, and aspirated. Liquid scintillation cocktail (0.5 mL; Packard Bell Ultima Gold) was added prior to radioassay in a liquid scintillation counter (Beckman LS 6000TA). To convert ^{3}H-Leu uptake into bacterial biomass, we used the theoretical conversion factor of [3], 1.0×10^{17} cells mol^{-1} ^{3}H-Leu incorporated and assumed a dilution factor of 2.

RESULTS

Bacterial productivity (^{3}H-Leu incorporation) was measured in and around the main GCW for shallow (Fig. 2) and groundwater samples in 1995 from April (Fig. 2(a); T1), August (Fig. 2(b); T2), November (Fig. 2(c); T2.5), and December (Fig. 2(d); T3). Production in all samples ranged from 880 to 7.7×10^4 cells mL^{-1} h^{-1} in the beginning of the experiment (Fig 2(a)) prior to increasing to 2.0×10^5 cells mL^{-1} h^{-1} in the main GCW in August (Fig 2(b)), 7.8×10^5 cells mL^{-1} h^{-1} in November (Fig. 2(c)) and, finally, doubling again to 1.6×10^6 cells mL^{-1} h^{-1} in December (Fig. 2(d); note scale change). Bacterial turnover times for the free-living assemblage decreased to around 1 h in December. Production increased

Production was also measured in the three biocurtain GCWs and the surrounding eight monitoring wells for shallow (Fig. 4) and deep (Fig. 5) samples for (a) T1, (b) T2, and (c) T3. Rates were always one to two orders of magnitude higher in the GCWs relative to the monitoring wells for the deep samples (Fig. 5). The same was observed for the shallow samples for T1 and T2 with the exception of MW-15, which may have had other nutrient influences (see Mueller et. al., Chapter I.1) (Fig. 4). At T3, two of the GCWs (200-1 and 200-2) showed dramatic increases in production (4.6×10^4 to 3.6×10^5 cells mL^{-1} h^{-1}), as did MW-15 (4.3×10^5 cells mL^{-1} h^{-1}) (Fig. 4).

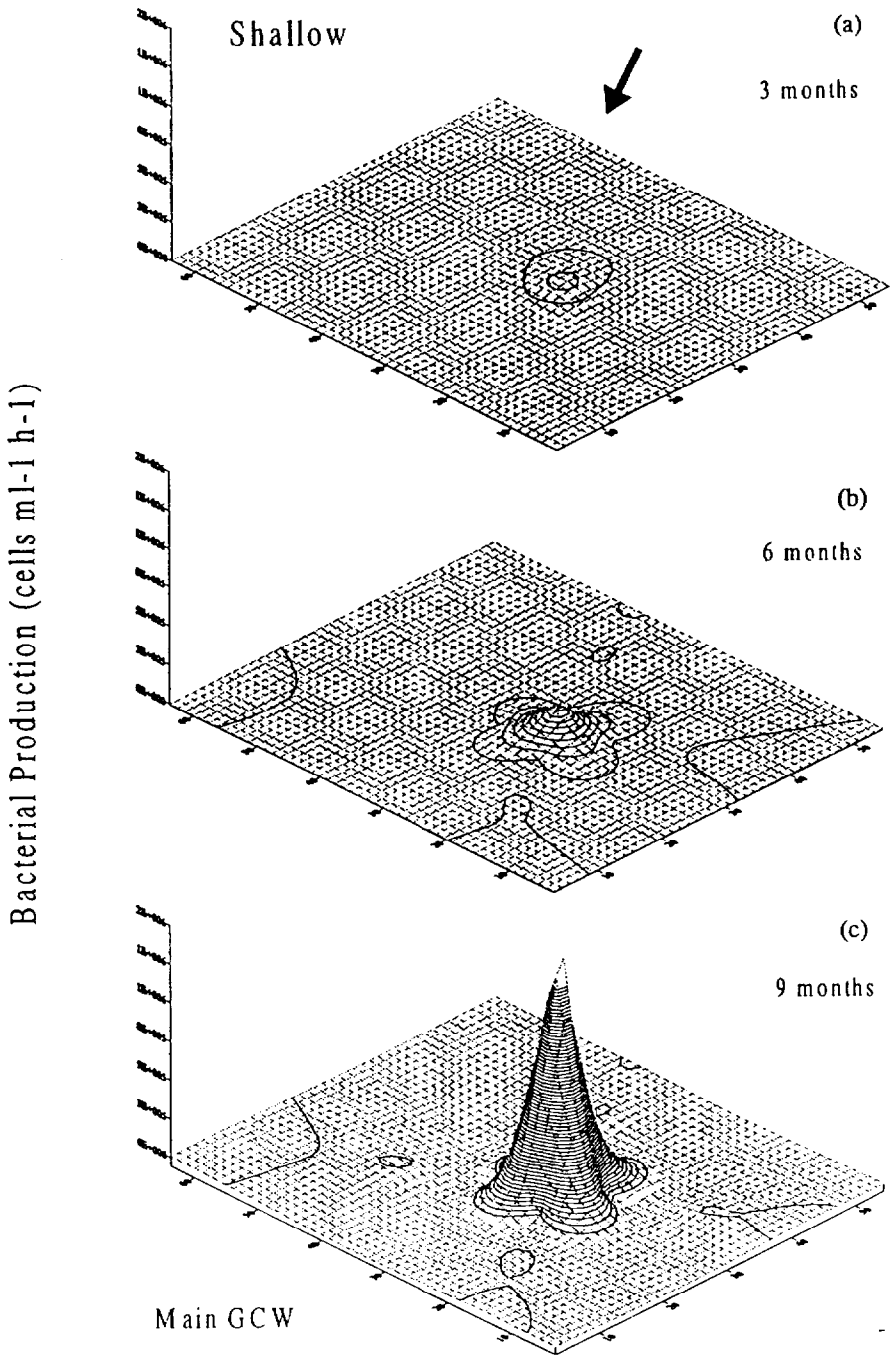

Fig. 2 — Bacterial productivity (cells h^{-1} ml^{-1}) measured in the main GCWs and 12 surrounding monitoring wells in the shallow samples for (a) April, (b) August and (c) December

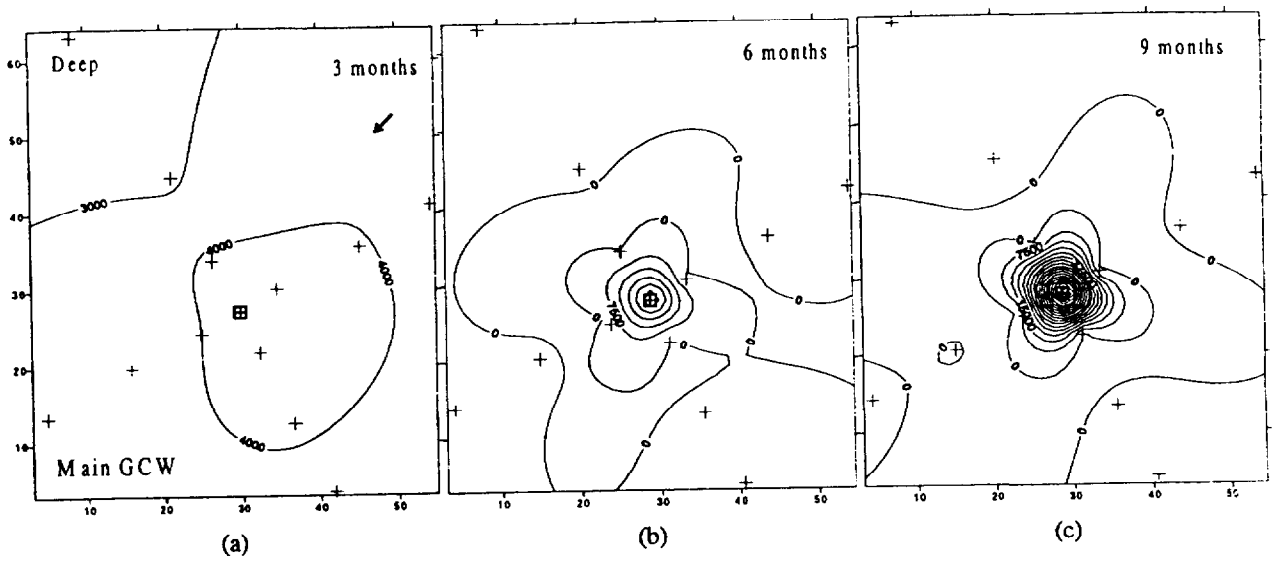

Fig. 3 — Bacterial productivity (cells h^{-1} ml^{-1}) measured in the main GCWs and 12 surrounding monitoring wells in the deep samples for (a) April, (b) August, and (c) December 1995

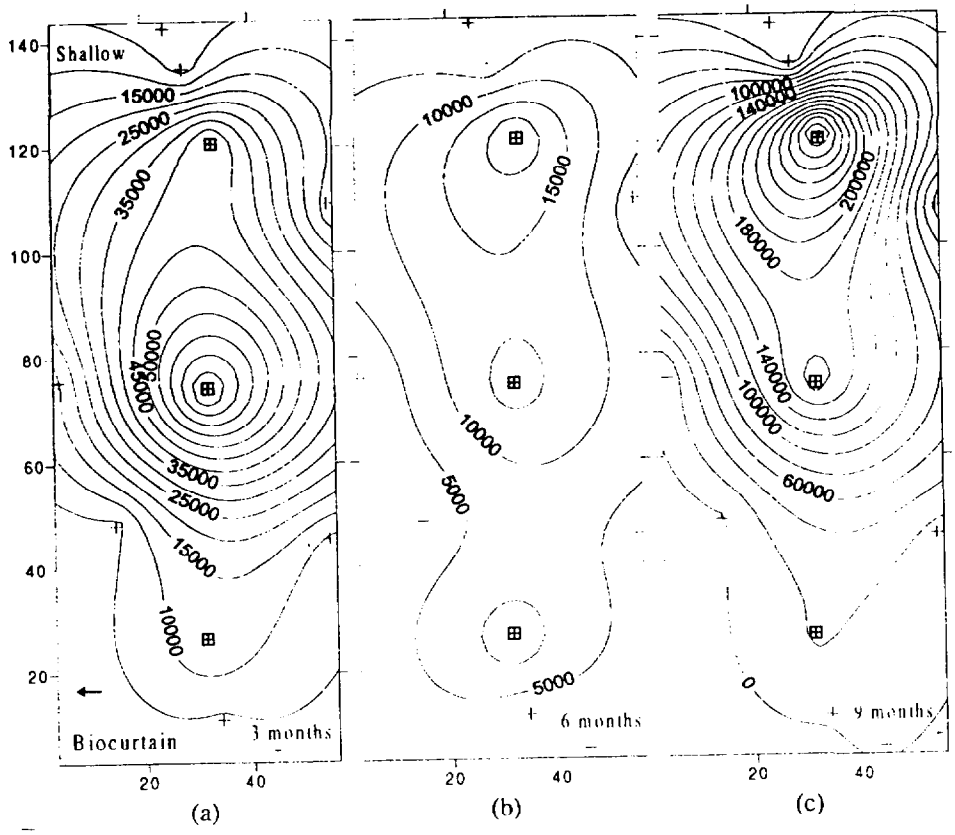

Fig. 4 — Bacterial productivity (cells h^{-1} ml^{-1}) measured in the three GCWs and eight surrounding monitoring wells in the shallow samples for (a) April, (b) August, and (c) December 1995

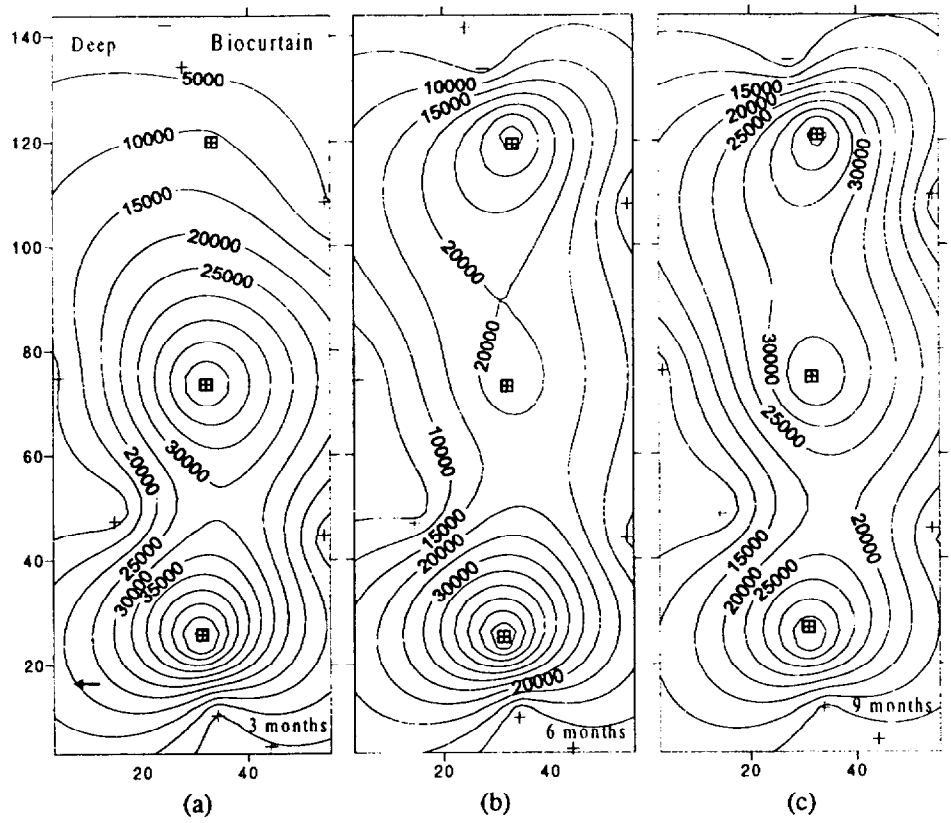

Fig. 5 — Bacterial productivity (cells h^{-1} ml^{-1}) measured in the three GCWs and eight surrounding monitoring wells in the deep samples for (a) April, (b) August, and (c) December 1995

DISCUSSION

GCWs may stimulate bacterial production by circulating limiting nutrients, enhancing bioavailability of hydrocarbons, or by removing inhibitory low-molecular-weight compounds through abiotic removal (air stripping). We found that bacterial production was stimulated across the zone of influence of a series of GCWs placed in an freshwater aquifer at a gasoline-contaminated site. Changes in bacterial abundance were not a useful parameter to evaluate stimulation of the natural assemblage resulting from the well operation, whereas productivity increased in response to a properly functioning well and decreased when well circulation was poor. Thus measuring instantaneous rates of heterotrophic production may provide a rapid assessment (< 1 d) of well performance.

Bacterial productivity increased from approximately 10^3 to over 10^6 cells mL^{-1} h^{-1} across the zones of influence of the main GCW and biocurtain, suggesting that this treatment stimulates overall biodegradation of carbon sources present in the groundwater. Production continued to increase over the 9-month experiment in the main GCW up to a rate of 1.6 cells mL^{-1} h^{-1}. This extremely rapid rate results in turnover time of the free-living assemblage of around 1 h, which is more typical of wastewater treatment plants than of groundwater environments. Productivity in the biocurtain was one order of magnitude lower and did not substantially increase until the final sampling. This may be due to low BTEX concentrations measured for these samples, thus the assemblage could be carbon-limited in the biocurtain groundwater samples.

REFERENCES

1. **Hobbie, J. E., R.J. Daley, and S. Jasper.** 1977. Use of nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225-1228.

2. **Kirchman, D. L.** 1993. Statistical analysis of direct counts of microbial abundance, p. 117-120. *In* P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole (ed.), Handbook of methods in aquatic microbial ecology. Lewis Publishers, Ann Arbor.

3. **Kirchman, D. L., E. K'Nees, and R. Hodson.** 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. **49**:599-607.

4. [illegible] synthesis rates in seawater using ^{3}H-leucine. Mar. Microb. Food Webs 6:107-114.